

# Safe Drinking Water Partnerships: Microbiology at the Technical Support Center

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## BACKGROUND

- The Office of Ground Water and Drinking Water (OGWDW) is responsible for developing and directing national policy for safeguarding national drinking water supplies from microbial and chemical contaminants.
- The Technical Support Center (TSC) of the OGWDW provides support to regulation development, regulation implementation, and analytical methods development and evaluation.
- These objectives are achieved by frequent collaborations between several U.S. Environmental Protection Agency (U.S. EPA) offices, water utilities, and academic institutions.
- Presented here are some of the collaborative efforts by the microbiology group of TSC.

## ENTERIC VIRUSES

### Introduction:

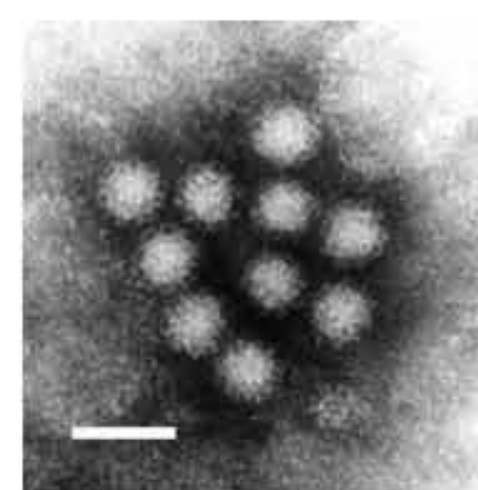
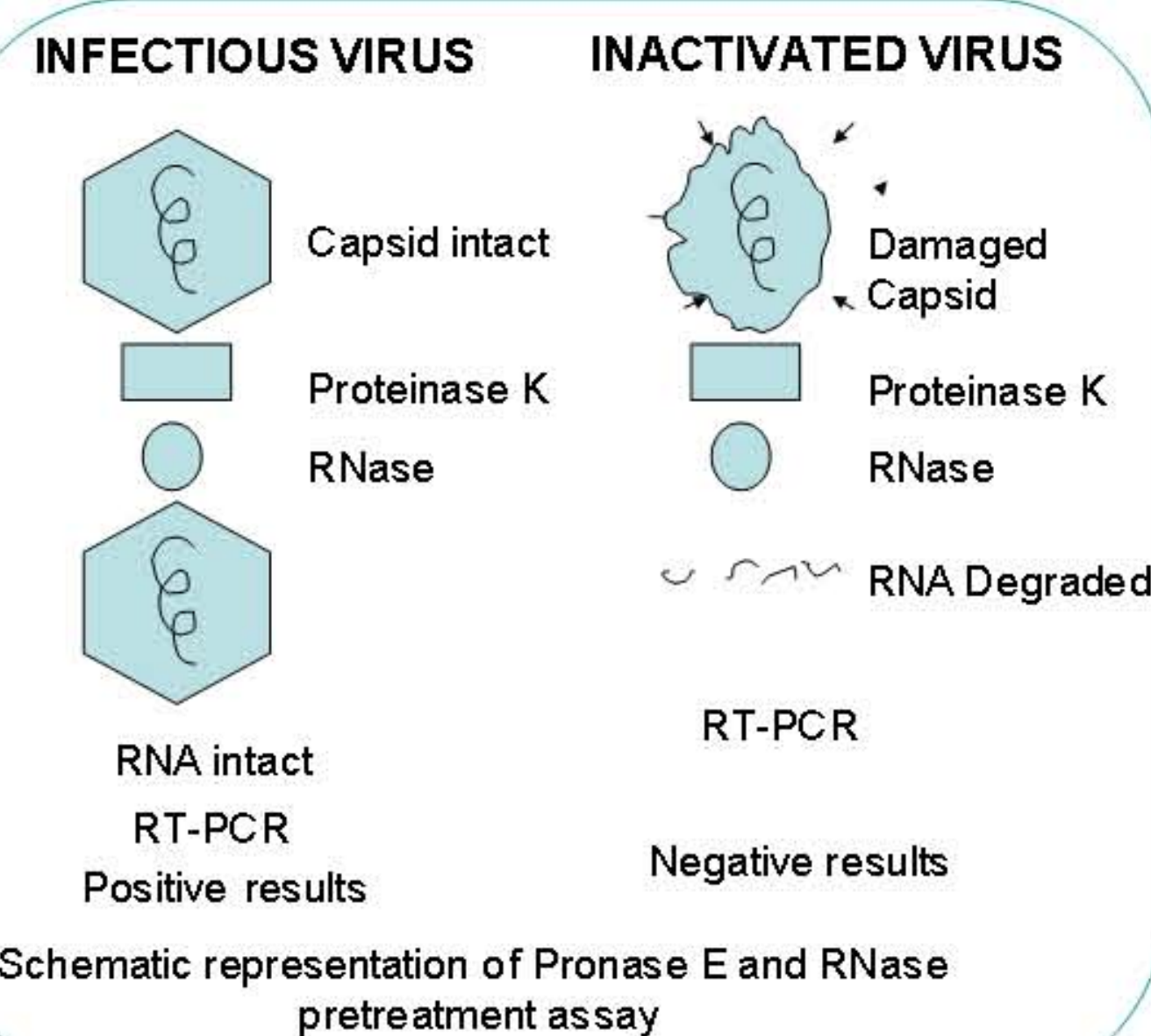
- Enteric viruses have caused several waterborne outbreaks.
- Current detection methods for waterborne enteric viruses cannot differentiate between infectious and non-infectious states in all viruses.
- An important public health issue is whether enteric viruses present in water samples are infectious.
- Goal of this project was to develop a rapid molecular method that differentiates between infectious and non-infectious viruses.

### Method:

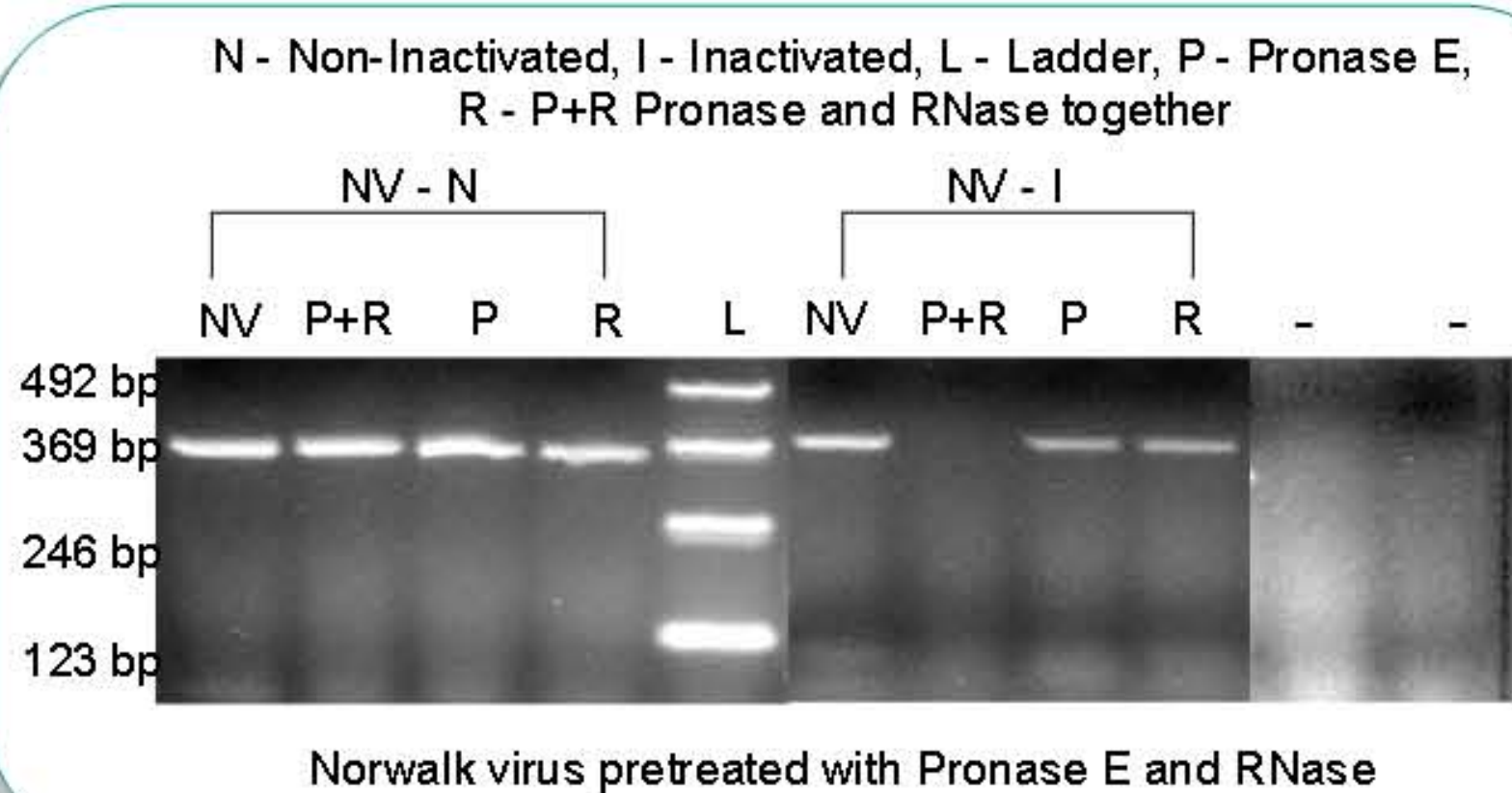
- Enteric viruses such as poliovirus, coxsackie virus, echovirus and Norwalk virus were inactivated by heat or chlorine.
- Inactivated and non-inactivated viruses were pretreated with Pronase E and RNase prior to Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).
- Virus nucleic acid was extracted.
- RT-PCR was performed and products were visualized on ethidium bromide stained gels.

### Results and Conclusions:

- Inactivated viruses yielded negative RT-PCR results while non-inactivated viruses yielded positive RT-PCR results when treated with Pronase E and RNase prior to RT-PCR.



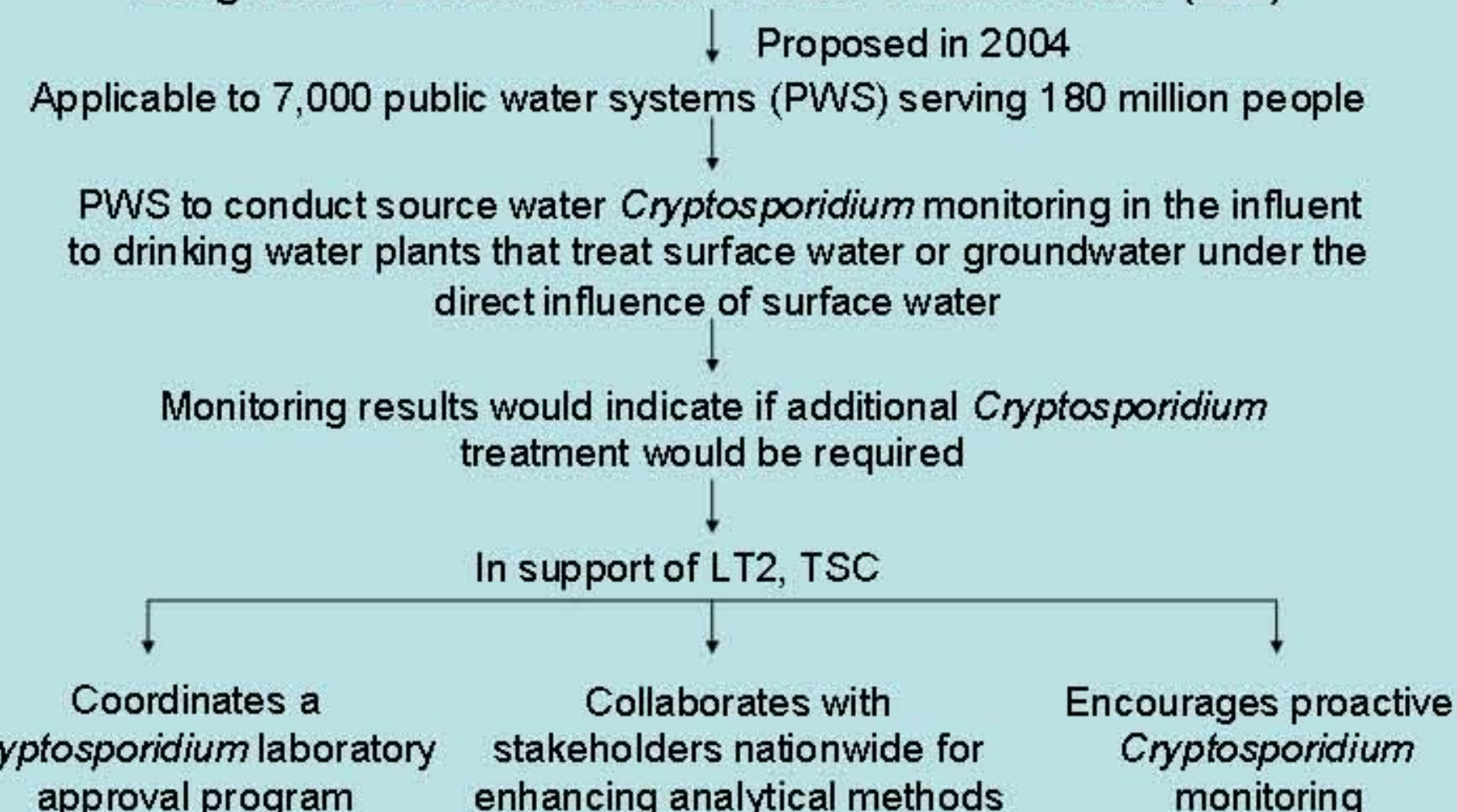
Norwalk virus



Norwalk virus pretreated with Pronase E and RNase

## CRYPTOSPORIDIUM MONITORING

### CRYPTOSPORIDIUM MONITORING: Long Term 2 Enhanced Surface Water Treatment Rule (LT2)



## AEROMONADS

### A. Small and Large scale monitoring of *Aeromonas*

#### Introduction:

- *Aeromonas* are autochthonous inhabitants of aquatic environments worldwide.
- *Aeromonas* are implicated as etiological agents in a variety of human diseases including gastroenteritis, wound infections, and septicemia.
- Goal of the project was to monitor for the presence of *Aeromonas* and to identify the different biotypes.

#### Methods:

- In a preliminary study, TSC collaborated with National Exposure Research Laboratory (ORD/NERL) to isolate and identify *Aeromonas* species from a small sample of drinking water distribution system supplies across the U.S.
- *Aeromonas* were also monitored for under the Unregulated Contaminant Monitoring Rule (UCMR) in 2003.
- 212 isolates that were obtained in the preliminary study were subjected to biochemical testing along with a Restriction Fragment Length Polymorphism (RFLP) analysis to type and identify the isolates to determine species.

#### Results and Conclusions:

- Several different biotypes of aeromonads were identified, including those that are suspected to be pathogenic to humans.
- *Aeromonas* species isolated from the UCMR study are being further characterized by strain identification.



Colonies of *Aeromonas hydrophila* on ADA-V agar Plate

### B. Virulence Factors of *Aeromonas*

#### Introduction:

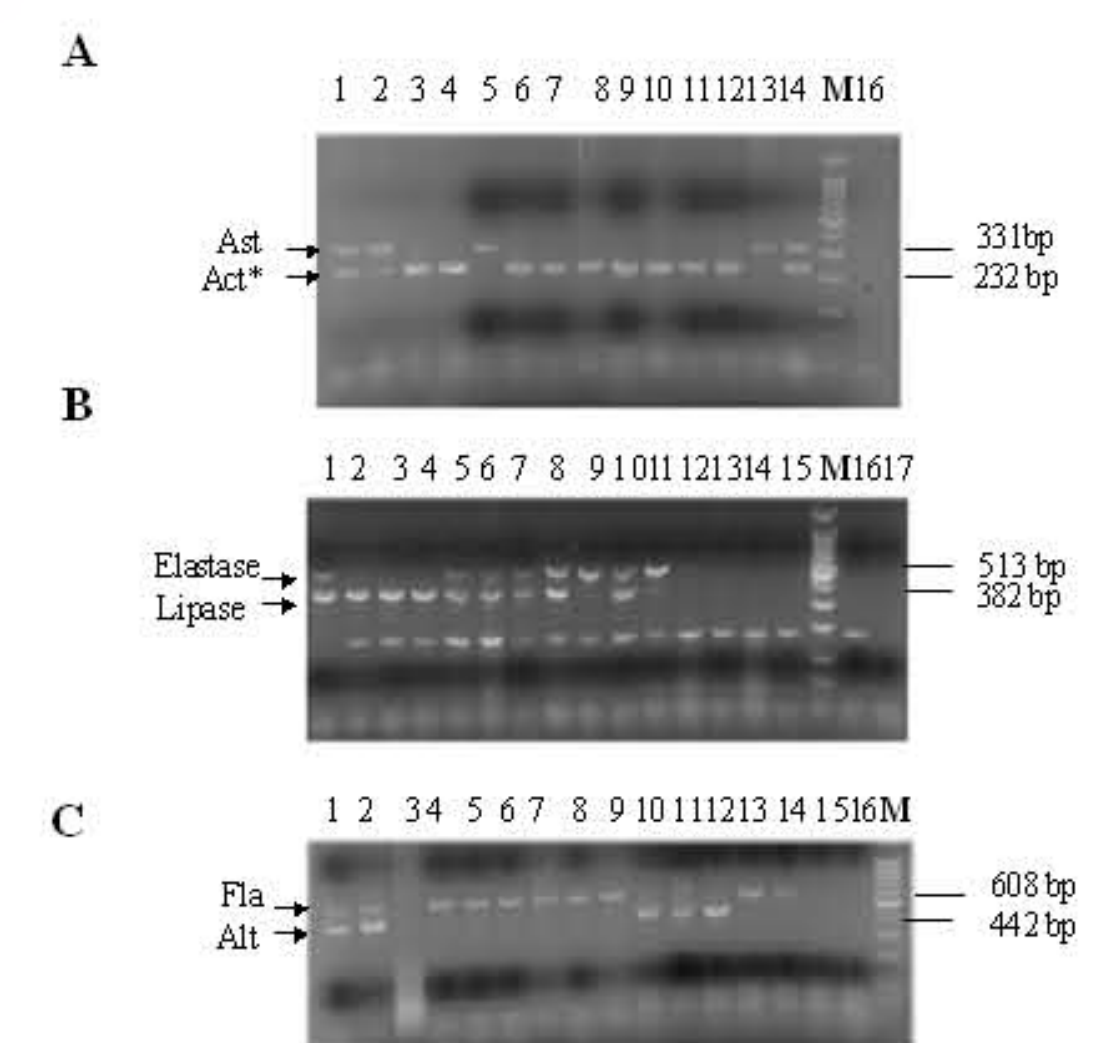
- The virulence of *Aeromonas* bacteria in causing diarrhea and other infections is generally believed to be multifactorial.
- An important concern to the EPA is whether *Aeromonas* species isolated from drinking water are potentially virulent.
- Goal of the project was to establish a method to characterize the virulence of *Aeromonas* isolates.

#### Methods:

- A genetic characterization of 8 virulence factor genes was performed using Polymerase Chain Reaction (PCR), with 52 drinking water isolates.
- Oligonucleotide primers to the genes, elastase (*ahyB*), lipase (*pla*), polar flagella (*flaA*/*flaB*, *flaG*), the enterotoxins *alt*, *act* and *ast*, and lateral flagella (*lafA*) were used in 4 duplex PCR assays.
- ORD/NERL performed animal model studies with immuno-compromised mice which were intraperitoneally injected with selected *Aeromonas* isolates.

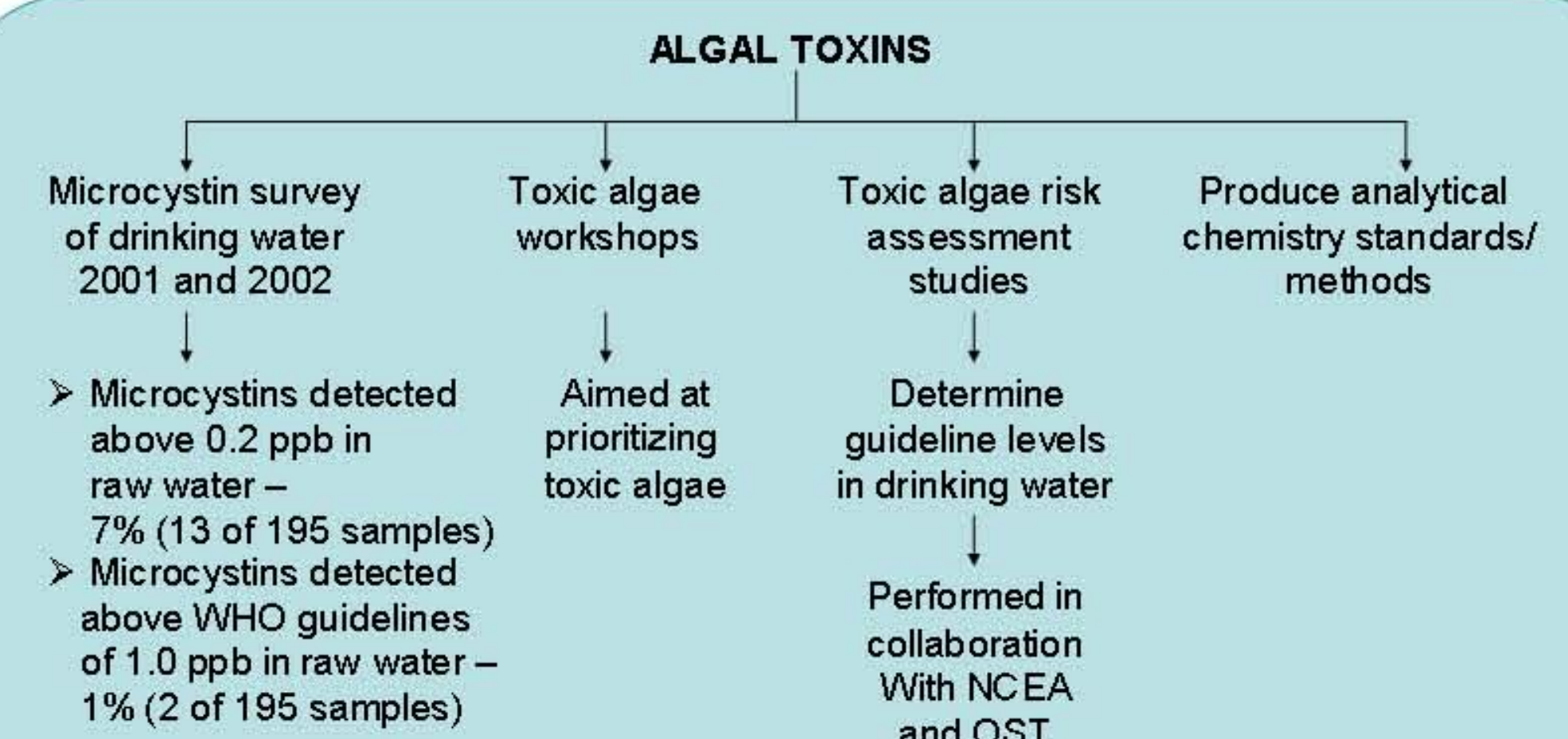
#### Results and Conclusions:

- The genes *ahyB*, *pla*, *flaA/B*, *flaG*, *act*, *alt* and *ast*, and *lafA* were present in 88 %, 88%, 67 %, 68%, 69 %, 46%, 32% and 44% of the isolates, respectively.
- Multiple species were isolated from most of the utilities and different combinations of virulence factors were observed in different strains of the same species.
- Isolates that had *lafA* or the lateral flagella gene, and one or more of the enterotoxin genes, showed clear signs of being virulent in animal studies.
- 90 % of *A. hydrophila*, 84 % of *A. veronii*, 100 % of *A. caviae* strains showed this correlation.
- The other strains were avirulent as expected because they are not normally pathogenic to humans.



Detection of Virulence Genes by Polymerase Chain Reaction (PCR)

## ALGAL TOXINS



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